molecular events that drive this modified expression of COMP in OA cells.

Methods: Human cartilage specimens were derived from the articular surface of patients undergoing total knee arthroplasty. Sampling was performed in different sites per each patient. Half of each sample was examined and graded histologically. Normal (N) or osteoarthritic (OA) cells were released from the remaining half by repeated enzymatic digestions. Cells were cultured in 10% serum-containing medium untill they reached 5 duplications. Cultures were included in the study when both N and OA cells were derived from the same subject. Four double cultures (N and OA) were obtained from four different donors. mRNA extraction, qualitative and Real Time RT-PCR were performed. A chromatin immunoprecipitation assay (ChIP) was used to detect the presence, on the COMP promoter, of the transcription factor Sox 9, a key regulator of chondrogenic differentiation. Maintenance of the chondrogenic potential was tested *in vitro* by means of high-cell density micromass culture; resulting pellets were processed for cytochemistry.

Results: A marked reduction (48%) of the COMP mRNA level was evidenced in OA samples, (panels A and B). ChIP analysis, on the same cells, revealed that the transcription factor Sox 9 was binding the COMP promoter only in the extracts (E) derived from the N chondrocytes, whereas it was absent in those derived from the OA cells. On the contrary, the DNA inputs of N and OA chondrocytes, before immunoprecipitation, provided the proper amplified PCR products (T+) when used as control templates (panel C). Contamporarily, a Sox 9 transcript was PCR-amplified in N and OA cells, indicating the factor's presence in both cell types (panel D). A decreased chondrogenic potential in OA cells was evidenced by the comparative staining of the matrix components in N and OA pellets (panel E).

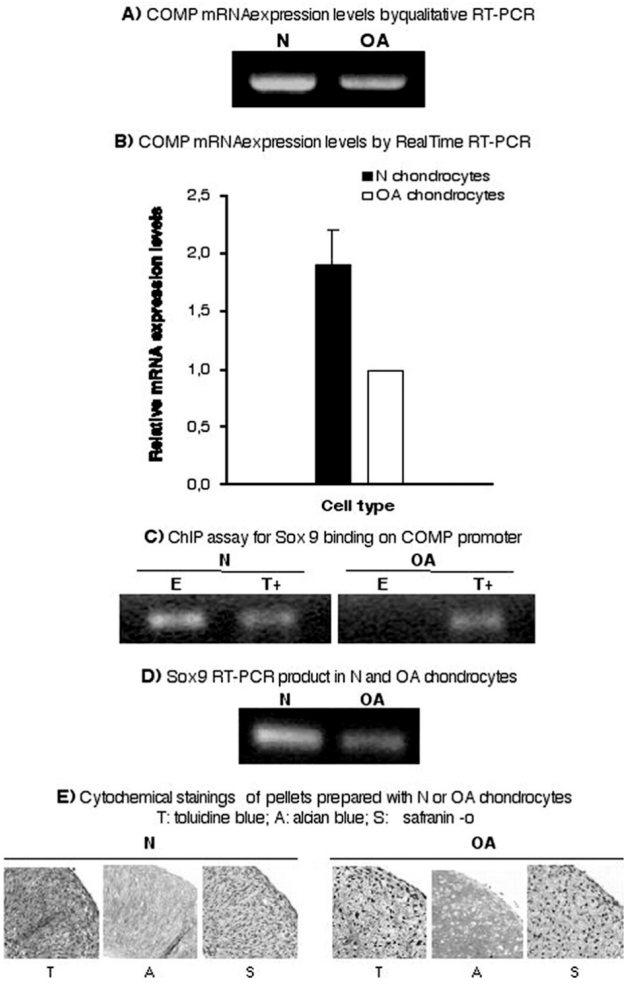

Fig. 1

Conclusions: The maintenance of the potential reversibility of the phenotypic changes in cultured chondrocytes is crucial to the cell-mediated cartilage resurfacing attempts. Indeed, if cartilage-specific molecular transactivators, such as Sox 9, are prevented from binding their responsive elements on target genes, any repair attempt that includes OA-affected cells becomes undermined. Putatively, displacement/sequestration mechanisms -such as those driven by IL-1 and the transcription factor C/EBP- could act either directly on Sox 9 or on other coactivator proteins, such as p300, reducing Sox 9 availability in OA cells, and ultimately minimizing their chondrogenic potential. Studies are currently being performed to verify this hypotesis.

A30

HYPOXIC EXPANSION OF ARTICULAR CHONDROCYTES PROMOTES THE FORMATION OF A HYALINE CARTILAGE-LIKE MATRIX IN MICROMASS CULTURES

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Purpose: Cartilage lesions in young patients will lead to premature osteoarthritis. Cell based repair strategies, such as autologous chondrocyte implantation and matrix assisted chondrocyte implantation, require an expansion phase to obtain the cell numbers required for therapy. Even though recent reports demonstrated that the function and differentiation of chondrocytes depend on O2 tension, the induction of chondrocyte differentiation only was assessed at low pO2. The potentially beneficial effects of low pO2 in the expansion of the cells, however, have not been considered, since most of the published data refer to normoxic conditions. The purpose of this study was to investigate the effect of different O2 tensions during the expansion of chondrocytes on their subsequent redifferentiation in high-density micromass cultures.

Methods: Bovine articular chondrocytes were grown to confluence in monolayer cultures at 1.5%, 5%, and 21% O2. Subsequently, micromass cultures were grown at 1.5%, 5%, and 21% O2. The metabolic status of the cells and the content in glycosaminoglycans (GAGs) were assessed biochemically. The expression of transcripts specific for chondrocytes was analyzed by real-time PCR. Histological assessment included in situ hybridization for collagen transcripts, immunohistochemistry, and safranin O staining.

Results: After expansion of chondrocytes mitochondrial citrate synthase (CS) and lactate dehydrogenase (LDH) were determined to assess aerobic and anaerobic energy metabolism, respectively. At 1.5% O2, proliferation and CS-activity were increased, whereas LDH-activity was decreased, as compared to 5% and 21% O2. Levels of mRNAs encoding collagen type I (col(I); 1000-fold) and aggrecan (2-fold) were increased while levels of transcripts encoding col(II) were decreased (5 to 10-fold) in all conditions. Micromass cultures were assessed for the synthesis of a cartilage-like matrix. Redifferentiation at 21% O2 led to higher contents in GAGs as compared to 5% and 1.5% O2, regardless of the preceding expansion conditions. After 14 days at 21% O2, the following observations were made: (i) col(II) mRNA levels nearly reached the levels detected in freshly prepared chondrocytes, whereas mRNAs encoding col(I) and aggrecan remained unchanged. (ii) The content of sulfated GAGs (DMB assay) and the histological appearance revealed no differences between micromass cultures arisen from cells expanded

at 5% and 21% O2. (iii) The content in sulfated GAGs was the same or increased in micromass cultures arisen from cells expanded at 1.5% compared to 21% O2, whereas the content of GAGs using a safranin O based assay consistently showed a significant increase. (iv) Micromass cultures from cells expanded at 1.5% O2 showed remarkably stronger staining for GAGs, with cells localized within lacunae, as compared to cultures from cells expanded at 5% and 21% O2. (v) The collagen type II positive matrix colocalized with cells expressing col(II) mRNA.
Conclusions: The lower CS- and the higher LDH-activity in cells expanded at 1.5% suggest that an anaerobic metabolism can be maintained during the expansion phase. These cells retain the capacity to develop a cartilage-like matrix in subsequent micromass cultures with respect to the content of GAGs. The data suggests that applying hypoxic conditions during the expansion phase of chondrocytes may improve the cartilage-like properties of cell-based constructs used for the treatment of cartilage lesions.

A31

BLOOD LEAD LEVELS ARE ASSOCIATED WITH MORE SEVERE RADIOGRAPHIC KNEE OSTEOARTHRITIS

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Purpose: Appreciation of the role of bone in the pathogenesis of osteoarthritis (OA) is gaining importance, but mechanisms involved in this process are unclear. Lead (Pb) exposure occurs throughout the lifetime and is primarily stored in bone. Pb is a skeleton toxicant. Toxicological studies have demonstrated adverse effects of Pb on bone formation and resorption by affecting both osteoclasts and osteoblasts. Emerging evidence also suggests chondrocytes as another target of Pb toxicity. Whether Pb exposure is involved in OA pathogenesis has not been explored. We examined associations between whole blood Pb levels and radiographic knee OA in African American and white men and women in the Johnston County Osteoarthritis Project.
Methods: Blood Pb levels of 790 adults (mean age 60.2 (10.4), 44% African American; 34% male) in the Johnston County Osteoarthritis Project Metals Exposure Sub-study were determined by inductively coupled plasma mass spectrometry (Inorganic Toxicology Laboratory of CDC-National Center for Environmental Health, Atlanta, GA). Radiographic knee OA (rKOA) was defined as Kellgren-Lawrence grade 2-4 using fixed flexion posterior-anterior knee films read by a single trained radiologist. Two rKOA variables were examined: 1) laterality (bilateral vs. unilateral/none) and 2) severity (moderate/severe vs. mild/none). General linear models were used to assess associations between rKOA variables and logarithmically-transformed Pb levels (lnPb), adjusting for age group (45-54, 55-64, 65-74, 75 years and older), gender, race, education (12+ years vs. $<$12 years), body mass index, current alcohol use and current smoking status (yes, no). Multiple logistic regression was used to examine associations between rKOA outcomes and blood Pb (in quintiles), adjusting for age group (45-54, 55-64, 65-74, 75 years and older), gender, race, education (12+ years vs. $<$12 years), body mass index, current alcohol use and current smoking status (yes, no). Interactions by race and by gender were tested, with p-values for an interaction term of $<$0.1 required for statistical significance.
Results: Median Pb levels were 2.0 (range 0.5 - 42). Adjusted least squares means for lnPb were higher in older age groups ($p = 0.001$), African Americans vs whites, (1.26 vs 0.87, $p < 0.0001$), men vs women (1.20 vs 0.93, $p < 0.0001$), those with less educational attainment (1.16 vs 0.97, $p < 0.0001$), current alcohol users (1.22 vs 0.91, $p < 0.0001$), current smokers (1.21 vs 0.92, $p < 0.0001$), those with bilateral rKOA (1.12 vs 1.00, $p = 0.03$), and those with severe rKOA (1.13 vs 1.00, $p = 0.01$). Compared to those in the lowest quintile, those in the highest quintile of blood Pb had a 60% increased odds of bilateral rKOA (aOR = 1.6 [0.7, 3.3]) and twice the odds of severe rKOA (aOR = 2.1 [1.0, 4.4]). There were no significant race or gender interactions.
Conclusions: Our cross-sectional analyses showed that increased blood Pb was associated with rKOA severity in African American and white men and women, supporting the hypothesis that environmental Pb exposure may be a novel and potentially modifiable risk factor for adverse OA outcome. Prospective studies are warranted to investigate the role of Pb in OA development and progression.

A32

DIETARY VITAMIN D INTAKE AND RADIOGRAPHIC KNEE OSTEOARTHRITIS

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Purpose: Low intakes of dietary vitamin D have been associated with an increased incidence of hip osteoarthritis and progression of knee osteoarthritis. There is however, little data on the association with patello-femoral joint osteoarthritis. The exact mechanism of this association is uncertain. The aim of this study was to assess whether dietary vitamin D intake was associated with the prevelance of radiographic knee osteoarthritis and to determine whether this association was due to effects on osteophytosis or joint space narrowing and also to explore associations with patello-femoral osteoarthritis.
Methods: This study was conducted using the MRC Hertfordshire Cohort Study of 957 men and women born between 1931 and 1939. They completed a detailed lifestyle questionnaire, including a formal food frequency questionnaire. They also had AP weight bearing and lateral non weight bearing radiographs of the knee. Radiographs were graded according to the Kellgren and Lawrence scale, but also separately for osteophytes and joint space narrowing using the Baltimore grading scale. Vitamin D intake was calculated from dietary sources and additionally from food supplements. Intake was divided by kilo calories to produce vitamin D dietary density.
Results: We studied 498 men and 459 women with the following characteristics: age 64.8 (60.5-69.1) and BMI 26.5 (21.3-31.7). 15.7% of patients had osteoarthritis defined by a K&L score of at

A32 – Table 1. Correlations of radiographic scorinng and dietary vitamin D intake

	Male and female		Male		Female	
	β	p value	β	p value	β	p value
Tibio-femoral K and L score	-0.150	0.028	-0.133	0.17	-0.169	0.08
Tibio-femoral Joint space score	-0.048	0.28	-0.050	0.17	-0.045	0.44
Tibio-femoral osteophyte score	-0.149	0.013	-0.104	0.21	-0.201	0.019
Patello-femoral K and L score	-0.057	0.36	-0.026	0.74	-0.093	0.34
Patello-femoral Joint space score	-0.025	0.66	0.017	0.83	-0.074	0.38
Patello-femoral osteophyte score	-0.112	0.069	-0.059	0.47	-0.172	0.063